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16. ABSTRACT

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Fate of Selected Herbicides in a Terrestrial Laboratory Microcosm

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■ The transport and metabolism of ^{14}C-labeled herbicides (simazine, bromacil, trifluralin, and 2,4,5-T) applied as a foliar spray (0.28 kg/ha) was examined in a terrestrial microcosm chamber (TMC). These chemicals were compared to a reference compound, the insecticide dieldrin. The TMC contained a synthetic soil medium, Douglas fir and red alder seedlings, rye grass, numerous invertebrates, and a gravid gray-tailed vole (*Microtus canicaudus*). By 20 days posttreatment, total soil residues (parent and metabolites and bound residues) averaged 0.14 ppm for all chemicals. Except for dieldrin little extractable parent material was detected for any of the chemicals in the soil. Concentrations of ^{14}C material in the rye grass shoots ranged from an average of 2.5 ppm for 2,4,5-T to 16.8 ppm for simazine. 2,4,5-T and trifluralin were more rapidly degraded than the other chemicals with 2,4,5-T present primarily as extractable metabolites. ^{14}C material of dieldrin was accumulated to a much greater extent than any of the herbicides in the invertebrates. While concentrations of all chemicals in the vole were low, ^{14}C material from dieldrin and simazine was present at levels approximately twice those of the other chemicals. None of the chemicals could be detected in the ground water.

Introduction

This is the third in a series of experiments in which representatives from broad categories of pesticides were examined in a terrestrial microcosm chamber (TMC) developed at the U.S. EPA's Corvallis Environmental Research Laboratory (CERL), as described earlier (*1,2*). Microcosms allow the investigator to examine materials that may be environmentally harmful with little risk to the environment and at a much reduced cost in comparison to field studies. The utility of a microcosm rests on its adaptability to studying a wide variety of chemicals in different types of ecosystems. The TMC has previously been used to study insecticides and fungicides in an agricultural ecosystem. In this study herbicide application in a nonagricultural system is examined.

Douglas fir reforestation practices have dictated the widespread use of selective herbicides to control red alder and

other deciduous trees in new growth areas. Such practices have recently generated widespread controversy about the possible adverse impacts of these chemicals primarily in relation to human health. The chemicals used in this study were herbicides commonly used in "conifer release", "right-of-way" control, or as general purpose (noncrop) herbicides. The specific chemicals selected were simazine (2-chloro-4,6-*bis*(ethylamino)-*s*-triazine), bromacil (5-bromo-3-*sec*-butyl-6-methyluracil), trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine), and the isooctyl ester of 2,4,5-T (isooctyl 2,4,5-trichlorophenoxyacetate). These were compared with the insecticide dieldrin (HEOD; 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4:5,8-*endo*,*exo*-dimethanonaphthalene), which serves as a reference compound in our system.

The primary objective of this experiment was to show that the fate and effects of chemicals applied to a "forest ecosystem" microcosm could be followed in the same manner and by the same techniques as an "agroecosystem" microcosm (*3*,*4*). The secondary objective was to determine the disposition of the various herbicides within the system and compare this information with that of the reference chemical.

Methods and Materials

Chamber. The terrestrial microcosm chamber (TMC) utilized in this study consisted of a glass box 1.0 × 0.75 × 0.60 m with a Plexiglass lid (*2*). The chamber received 2500 ft cd of light at the soil surface with a 16-h daily photoperiod. Temperatures ranged from 25 °C during the day to 15 °C at night. The airflow rate was 50 L/min. The soil medium consisted of 40% 20-grit washed sea sand, 40% illite clay, and 20% Jiffy Mix Plus (George Ball Pacific Incorp., Sunny Vale, CA).

Flora and Fauna. Plants consisted of a Douglas fir seedling (*Pseudotsuga menziesii*) in each quadrant surrounded by red alder seedlings (*Alnus rubra*) and perennial ryegrass (*Lolium perenne*). The fir were approximately 1 yr old (20 cm high) at the time of planting while the red alder were 2 months old (6 cm high).

Invertebrates were represented by bacteriophagic nematodes (*Pristionchos inheriteiri*), earthworms (*Lumbricus* spp.), pillbugs (*Armadillarium* and *Porcellia* spp.), mealworm larvae (*Tenebrio molitar*), gray crickets (*Achetus domesticus*), and garden snails (*Helix pomata*). The lone vertebrate was a gravid female gray-tailed vole (*Microtus canicaudus*).

Radiolabeled Material. The ^{14}C-dieldrin (labeled in positions 1, 2, 3, 4, and 10) was technical grade (>85% HEOD) obtained from Amersham Searle (Arlington Heights, IL). It was purified by preparative thin-layer chromatography to >98%. Bromacil (2-^{14}C, >96%), simazine (ring-^{14}C, >96%), trifluralin (ring-^{14}C, >97%), and the isooctyl ester of 2,4,5-T (ring-^{14}C, >94%) were obtained from New England Nuclear (Boston, MA) and used without further purification.

Procedure (3,4). The soil was added to each of the six chambers in three 40-kg portions. Following the addition of each layer, 6 L of water was added and the layer allowed to compact overnight. When all three layers had been added, the boxes were warmed to 30–35 °C for 1 week, after which time airflow was started; the temperature was adjusted to operational levels (15–25 °C) and a 16-h light cycle was initiated. One Douglas fir seedling was planted in the center of each quadrant and surrounded by four red alder seedlings. Forty grams of rye grass seed were scattered over the surface and covered with approximately 1 cm of soil mixture. Water was added to each TMC via an overhead sprinkler system three times a week (15 L total/week). After rye grass growth was observed, populations of earthworms and mealworm larvae were added, followed by snails and crickets. Twenty-six days after planting, five microcosms were treated with one of the test chemicals with the sixth chamber serving as a control. Application was by foliar spray at a rate of 0.28 kg/ha (0.25 lb/a). Thus each TMC was treated with 20 mg of material containing 4.0×10^7 dpm of ^{14}C. Treatment solutions were made by dissolving the agents in small amounts of acetone, adding the carrier normally used with the pesticide, and mixing thoroughly. The control was treated with xylene only (which served as the carrier for the dieldrin treatment). Eight days after treatment the vole was introduced.

A census of visible animal species was conducted daily. Dead animals were removed as found. Polyurethane air filters were changed and analyzed three times on the day of treatment and daily thereafter. Rye grass was sampled for analysis on days 2, 3, 6, and 13 posttreatment and at termination. The termination days for bromacil, simazine, trifluralin, 2,4,5-T, and HEOD were 22, 26, 19, 20, and 20 days after treatment, respectively.

At termination, the macrobiota (flora and fauna) were removed, counted, weighed, and frozen until analysis. A grid was created on the soil surface which comprised 50 10 × 15 cm units. From this grid 26 designated units were removed from the first 5-cm layer for individual residue analysis. This same pattern was applied to the second 5-cm layer. Sampling in levels 3 and 4 involved pooling of the units so that 13 and 6 samples were obtained, respectively. Following removal of all soil samples, the interior surfaces of the chambers were cleaned extensively with acetone and ethanol to remove all residual radioactivity.

Analysis. Water samples were extracted in a separatory funnel with acetone; soil samples were extracted by blending with hexane/isopropyl alcohol (3:2); plant tissue was extracted by blending with acetone; and animal tissue was extracted by blending with hexane/isopropyl alcohol and then acetone. Extract was separated from sample residue by using a Büchner funnel under vaccum. Following extraction all sample residues (except water) were combusted on a Packard sample oxidizer. All extracts and residues were analyzed for ^{14}C with a Packard Tri-Carb liquid scintillation spectrometer using commercially available counting cocktails. The ^{14}C activity of any sample was assumed to be the sum of the activities for all extracts plus combusted residue. If an organic extract of soil, plant, or animal tissue contained in excess of 10^3 dpm, the sample was further analyzed by thin-layer chromatography (TLC) and autoradiography. TLC was used to identify the presence of metabolites; however, identification of specific metabolites was not attempted.

Results

Mass Balance. Table I shows the total ^{14}C recovered for each chemical. Recovery values ranged from 69% for bromacil to 100+% for dieldrin. Overall recoveries were improved from previous tests of the TMC.

Recovery of HEOD exceeded even that of seed treatment (99%) (*4*). Figure 1 illustrates the comparative distribution within each TMC at termination of the experiment. Previously the soil was the major repository for ^{14}C (*3*,*4*); however, the plants generally contained greater amounts of ^{14}C in comparison to the soil in this study.

Residue Distribution. Soil. Concentrations of ^{14}C materials in the upper 5 cm of soil (Figure 2) averaged 0.14 ppm equivalents (bromacil, 0.12; simazine, 0.18; trifluralin, 0.08; 2,4,5-T, 0.19; HEOD, 0.12). In an earlier test with HEOD (0.25 lb/acre) applied as a seed coating, the average concentration of ^{14}C material in the upper layer exceeded 1 ppm (*4*). Except for HEOD, comparatively little extractable parent material was detected for any of the chemicals in the soil. HEOD, the least soluble of the chemicals examined, exhibited the greatest vertical distribution within the soil column.

Table I. Mass Balance ^{14}C (10^6 dpm) at Termination of TMC

	bromacil	simazine	trifluralin	2,4,5-T	HEOD
amount applied	39.9	39.4	43.4	37.3	40.5
amount recovered	27.6	31.3	31.4	34.1	41.8
% recovered	69	79	72	91.0	103.2
unaccounted dpm[a]	12.3	8.1	12.6	3.2	0

[a] May reflect the conversion of the ^{14}C-labeled material to $^{14}CO_2$, which was not detectable by the filtering apparatus.

Plants. Concentrations of ^{14}C material in the rye grass shoots ranged from an average of 2.5 ppm for 2,4,5-T to 16.8 ppm with simazine (Figure 3). Only 2,4,5-T was detected in the roots. The ^{14}C material of bromacil and simazine was present mainly as extractable parent, with some extractable metabolites. More extractable metabolites were found in the trifluralin treatment. 2,4,5-T was present almost exclusively as extractable metabolites, whereas HEOD was present as extractable parent. The red alders were not analyzed for either ^{14}C or pesticide residues because of extensive herbivory by both the vole and the crickets. Although the fir did receive some damage, sufficient plant material was present to permit analysis.

The fir trees accumulated relatively little of the ^{14}C material, although a proportionally greater amount was detected in their root system in comparison to the rye grass root system. The ^{14}C material from bromacil was found primarily as parent in the root, whereas it was generally present as extractable metabolite in the shoot. Extractable parent was not present for any of the other chemicals, with HEOD present only as a bound residue (material unextractable by solvent extraction; Figure 4).

Invertebrates. The ^{14}C material of HEOD was accumulated to a much greater extent in crickets, snails, and pillbugs than any of the herbicides (Table II). The snails generally exhibited greater concentrations of all chemicals compared to the other invertebrates, with the ^{14}C material of simazine and trifluralin being accumulated to the greatest extent. Extractable parent was detected for bromacil, trifluralin, and HEOD in the snail and for bromacil only in the cricket. ^{14}C-simazine material was present primarily as bound residues; the other chemicals were mainly aqueous metabolites. Snail feces collected from the glass sidewalls of the microcosm contained high concentrations of all chemicals. The vast majority of this material was detected as bound residues for all chemicals except dieldrin where parent material predominated. While condensate frequently occurred on the sidewall, relatively little ^{14}C activity was detected from wall swipes, thus minimizing the possibility of condensate adding to the feces ^{14}C activity. The high concentrations in the snail feces probably reflects intense foraging on the rye grass at night followed by prolonged stationary periods on the sidewalls during the day were the feces were concentrated.

Vole. Residues of ^{14}C in the vole ranged from 0.18 ppm equivalents for bromacil to 0.59 ppm for simazine (Table III). Extractable parent was detected only for HEOD. The other chemicals were present primarily as bound residues with the exception of 2,4,5-T which occurred primarily as extractable metabolites. Of the tissues examined (GI, heart, lung, liver, kidney, brain, and carcass), the gastrointestinal (GI) tract contained the vast majority of ^{14}C material for bromacil, trifluralin, and 2,4,5-T. Although ^{14}C simazine material was found primarily in the GI tract, the liver contained in excess of 2 ppm, mainly as bound residue. HEOD was found as extractable parent, metabolites, and bound residue in the liver and kidney, with the parent predominating. Total HEOD in the liver reached a level of 15.7 ppm, and 14 ppm in the kidney. The effects of the chemicals on the vole and the crickets and their interaction will be discussed elsewhere.

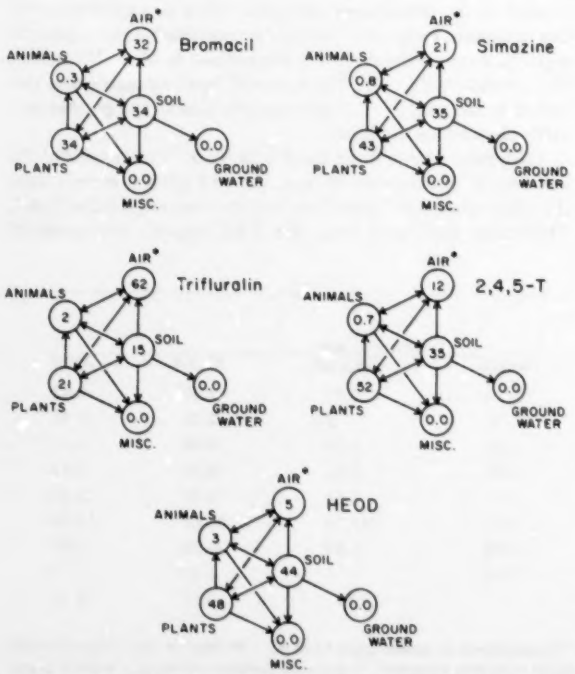

Figure 1. ^{14}C Mass balance (% of applied) in TMC at termination. *Amount measured plus estimated loss as $^{14}CO_2$.

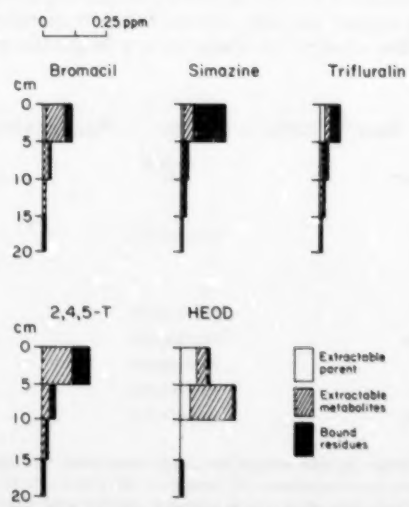

Figure 2. Vertical profile of terminal ^{14}C distribution in soil.

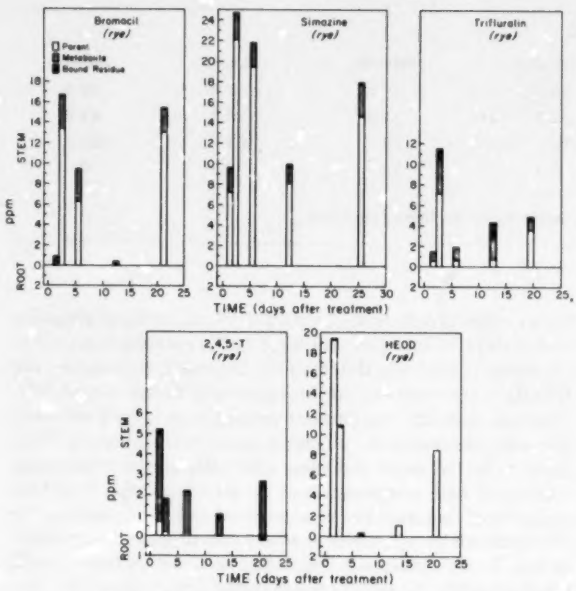

Figure 3. Profile of ^{14}C materials in rye grass.

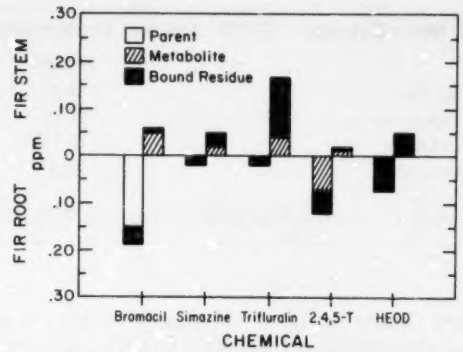

Figure 4. Profile of ^{14}C materials in Douglas fir seedlings at termination (bromacil = 22; simazine = 26; trifluralin = 19; 2,4,5-T = 20; HEOD = 20 days after treatment).

Air. Figure 5 represents only the ^{14}C trapped on the polyurethane foam air filters. Initial volatility losses of trifluralin greatly exceeded those of the other chemicals, with HEOD having the second highest loss rate followed by 2,4,5-T and a much more variable bromacil and simazine. By day 7 HEOD loss rate was relatively stable, whereas the others continued to decline. At termination, 5×10^5 dpm of ^{14}C from HEOD was still being trapped daily in the airstream.

Discussion

This study provides additional evidence that a simulated ecosystem such as the TMC can be used to indicate distribution trends of a wide variety of chemicals. In addition, comparisons between sets of experiments are possible even though conditions may differ. The recovery of ^{14}C-HEOD exceeded that of previous foliar treatments (*3*), because of improved air trapping capability with the polyurethane foam filters. The distribution of HEOD in this experiment is consistent with results from earlier work (*3,4*), with HEOD remaining at the site of application. With respect to HEOD metabolism in the soil, specifically low levels of extractable parent, we offer the following suggestions. Comparisons between microcosm and field studies cited in the literature are frequently difficult because of the minimal information available on the environmental conditions (e.g., temperature, light cycle, humidity, wind velocity) of the field studies. It is also possible that the relatively low airflow in combination with high temperature and humidity could accelerate metabolism. Furthermore, because of the conditions described above plus the organic matter content of the soil (5 wt %), the soil could be very active.

Recovery of bromacil, simazine, trifluralin, and 2,4,5-T was within an acceptable range in comparison to previous TMC experiments. Distribution of bromacil was complicated by placing the ^{14}C label in the second carbon position which is readily converted to $^{14}CO_2$. ^{14}C-bromacil material appeared to be evenly distributed between soil, plant, and air (Figure 1); however, the ^{14}C in the air probably reflects $^{14}CO_2$ rather then parent or volatile metabolite. Contrary to the findings of Hill (*5*) and Helling et al. (*6*), bromacil was relatively immobile in the soil, with the majority of the ^{14}C material residing in the upper 6 cm.

Based on a low vapor pressure (6×10^{-9} mmHg at 20 °C) and a solubility of 2 ppm, simazine should remain in the soil and the plant foliage (*7*). In a warm, moist climate such as existed in the microcosm, simazine could be expected to be less persistant in the soil, perhaps accounting for the relatively high ^{14}C levels in the air (*8*). In comparison to Metcalf's system (*9*), substantially more ^{14}C material from simazine was detected in the plant tissue, presumably due to the greater relative abundance of plants.

Trifluralin [vapor pressure of 1.99×10^{-4} mmHg at 29.5 °C (*7*)] would be expected to volatilize to a greater extent then the other chemicals tested, as was the case within the TMC. Trifluralin loss rates from the TMC appear substantially

Table II. Total[a] Pesticide Residue in Macroinvertebrates

species[e]	sample day[b]	chemical, ppm				
		bromacil	simazine	trifluralin	2,4,5-T	HEOD
cricket	7	0.67	0.96	0.41	1.30	1.42
	termination[c]	*d*	*d*	*d*	0.13	0.14
snail	8	0.52	2.40	1.70	0.88	2.41
	15	0.20	1.62	2.38	0.78	2.64
	termination	0.14	1.97	1.54	0.57	2.43
snail feces	termination	23.77	9.70	61.72	34.67	21.34
pillbug	termination	0.18	0.66	0.61	0.55	1.57
tenebrio	termination	0.11	1.04		0.23	*d*
earthworm	termination				0.32	0.09

[a] Total residue includes extractable parent, metabolites, and bound residue as ^{14}C equivalents of parent (μg/g fresh wt). [b] Number of days after treatment. [c] Termination day for bromacil = 22; simazine = 26; trifluralin = 19; 2,4,5-T = 20; HEOD = 20 after treatment. [d] No sample available. [e] Pillbugs, *Tenebrio*, snails, and earthworms were added prior to treatment; crickets were added prior to treatment, on the treatment day, and thereafter as needed.

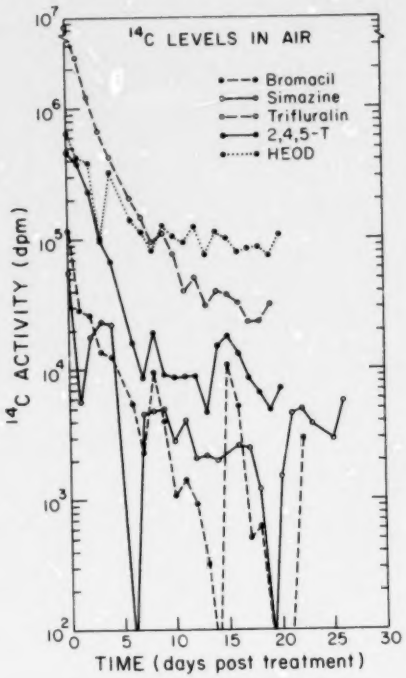

Figure 5. ^{14}C concentration in air.

greater than those observed in the field from a soil treatment (*10*), where 80–85% remained in the soil after 4 weeks in comparison to 15% in the TMC. Presumably the difference is due to the method of application.

The isooctyl ester of 2,4,5-T behaved as expected in the TMC with the majority of the ^{14}C material residing in the plants; however it was quickly degraded (Figure 3). The second most important repository was the soil, wherein again 2,4,5-T appears to be rapidly degraded (Figure 2), corresponding to results observed by McCall et al. (*11*). Metcalf et al. (*9*) observed similar results with respect to the plant/soil portion of his microcosm, although the plant material in the TMC contained substantially more ^{14}C materials from 2,4,5-T.

Conclusions

The following conclusions are based on the data from this experiment:

(1) The terrestrial microcosm chamber can be used to study the mass balance of herbicides in a terrestrial ecosystem (Figure 1).

(2) In general, distribution patterns observed in the TMC are comparable to those observed in other systems and field observations. However, insufficient information on the environmental conditions of the field studies can make comparisons difficult.

(3) In comparison to the reference compound (HEOD) all of the herbicides were less persistant in the TMC (Table I).

Table III. Whole Body Residues in Vole

	parent,[a] ppm	metabolites[b]	bound residue[c]	total
bromacil	nd[d]	0.08	0.11	0.18
simazine	nd	0.06	0.53	0.59
trifluralin	nd	0.06	0.20	0.26
2,4,5-T	nd	0.13	0.07	0.20
HEOD	0.4	0.06	0.01	0.47

[a] Extractable parent. [b] Polar metabolite(s), aqueous soluble material, and TLC origin material. [c] Bound residue as ^{14}C equivalents of parent (mg/g fresh wt). [d] None detected (<0.01 ppm).

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